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Microscopic Observations of the Digestion Condition of Pollen Grains in the Midgut of Stingless Bee Larvae

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Abstract.—The present paper presents results on the observations of the mechanisms of pollen digestion by larvae of stingless bees. The midgut content was observed with a transmission electron microscope. The morphological results suggest that pollen digestion in larvae can be a complex mechanism, which involves physical and chemical changes in the pollen cover by enzymatic action that may be allow some digestive enzymes to enter inside the pollen grains. Later, the intine may be ruptured as a response to the changes in the osmotic pressure, releasing the protoplasm for further digestion.

INTRODUCTION

The stingless bees, like other pollenophagous insects, are important pollinators of plants. Pollen is the major source of proteins, lipids and vitamins for both adults and larvae. Relatively little is known about how pollen grains are digested in the digestive tract of the bees.

In adult bees, there are many probable strategies used to digest pollen grains. Some authors have suggested that adult bees can break the pollen grains by chewing them, or by proventriculus action (Morton 1950, Snodgrass 1956, Von Planta 1985). On the other hand, Martinho (1975), Kroon *et al.* (1984) and Velthuis (1992) have pointed out that pollen grains can be digested only after an osmotic shock that takes place inside the digestive tract that causes the release of its content. However, Cruz-Landim (1985) and Cruz-Landim and Serrão (1994) suggested that digestion occurs before the content extrusion, therefore still inside the covers. Klungness and Peng (1984a, 1984b) suggested that pollen digestion can occur by different mechanisms, which depends on the species of pollen.

Data about digestion of pollen grains by the larvae are not available. Since all previous studies were made with adult bees, this study was conducted to investigate the digestion condition of pollen grains ingested by larvae of two species of stingless bees.

MATERIAL AND METHODS

Larvae were obtained from worker brood cells of the colonies of *Scaptotrigona postica* Latreille, 1807 and *Trigona spinipes* (Fabricius, 1794).

The digestive tracts of the larvae were removed into buffered saline solution for insects, and the midguts were isolated. The pieces were fixed in 2.5% glutaraldehyde in 0.1M Na cacodylate buffer at pH 7.2, washed twice in the buffer, post-fixed in 1% osmium tetroxide in the same buffer, dehydrated in a series of increasing concentrations of ethyl alcohol, and embedded in Epon-Araldite resin, following usual procedures. Thin sections cut with glass knives were stained with uranyl acetate and lead citrate, and examined in a Zeiss EM9S2 electron microscope.

RESULTS

At the anterior midgut of the larvae, the walls and protoplasm of pollen grains ap-

peared to be largely intact (Fig. 1). By the time the pollen reaches the posterior midgut, their contents were disorganized and gradually removed through the germination pores mostly without rupture of the pollen wall, forming an electron-lucent periphery (Figs. 2, 3).

Some pollen grains were seen with their contents disorganized and retracted in the center, leaving a clear ring around it (Fig. 4). In addition, material similar to the content of pollen grains were seen outside of them and appeared to be formed by many lipid droplets surrounded by electron dense material (Fig. 5). Broken pollen grains are rarely seen inside the midgut of the larvae.

DISCUSSION

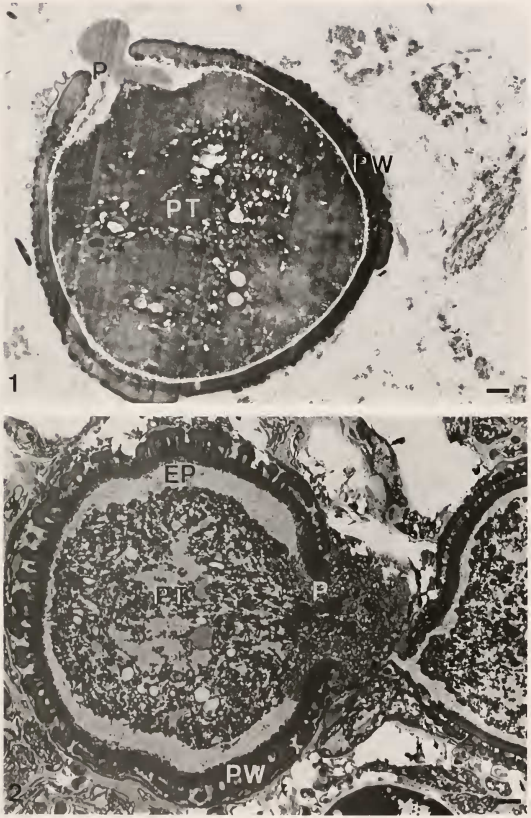
The absence of broken pollen grains in the larval midgut is an expected result, because larvae do not have strong mandibles or a proventriculus bulb, which break some pollen grains as pointed out by Morton (1950), Snodgrass (1956) and Von Planta (1985) for adult bees. The presence of pollen grains with their walls intact and with disorganized content suggest that pollen grains can be useful to larvae without their walls being broken. Similar results were observed in adult stingless bees by Cruz-Landim (1985) and Cruz-Landim and Serrão (1994).

Kroon *et al.* (1974) and Martinho (1975) have pointed out that since pollen grains are immersed in honey or nectar in the crop of the adult worker, the concentration of sugar of the pollen grains is altered. When these pollen grains enter the midgut, where sugar concentration is lower, they absorb water osmotically, so that the protoplasm is forced to protrude through the germination pores. Larvae do not have a crop where pollen and honey could be mixed inside the gut. The provisions of food for them are placed by nurse workers into the brood cells in layers consisting of (from bottom to top) by pollen, a mixture of honey and pollen, and glandular secretion (Sakagami *et al.* 1965, Sakagami and Zucchi 1966). Therefore, the mixture of pollen and honey is made in the brood cell. However our results never show protoplasm extrusion of the pollen grains in the anterior midgut. Therefore the model for pollen digestion suggested by Kroon *et al.* (1974) and Martinho (1975) for adult workers, above mentioned, has not morphological support to occurs in larvae.

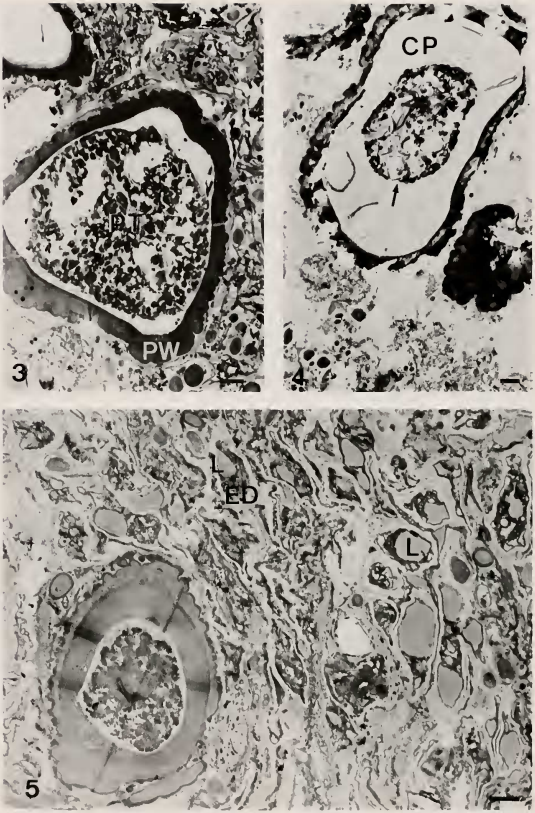
On the other hand, pollen grains in the middle and posterior midgut have the protoplasm released through the germination pore. Similar results were presented for adult *Apis mellifera* Linnaeus by Klungness and Peng (1984a) and Peng *et al.* (1986). These authors suggested the occurrence of enzymatic degradation of pectic acid and hemicellulose of the exine in the germination pores where the pollen wall is thin. As a result of these changes, the protein constituents of the intine are exposed to the digestive protease, causing the protoplasm extrusion. However, our morphological results show that before protoplasm extrusion, it is disorganized, suggesting that digestion begins inside the pollen wall, perhaps by action of enzymes that enter the grain through the permeable wall, as suggested to occurs in adult workers by Cruz-Landim (1985) and Cruz-Landim and Serrão (1994) or by pollen protease as thought by Grogan and Hunt (1979).

Therefore, we suggest that pollen digestion in stingless bee larvae can be a complex mechanism, which involves physical and chemical changes in the pollen cover by enzymatic action, which would permit that digestive enzymes enter into the pollen grains. Later, the intine may be ruptured, as a response to the changes of osmotic pressure, releasing the protoplasm for further digestion.

Presence of material similar to pollen grains but outside of them has been reported by Klungness and Peng (1984b) and Peng *et al.* (1986) as being of pollenkitt



Figs. 1–2. Fig. 1, *Trigona spinipes*. Pollen grains inside of the anterior midgut showing the pollen wall largely intact (PW). P—germination pore, PT—protoplasm. Bar = 1 μm. Fig. 2, *Scaptotrigona postica*. Pollen grain inside of the posterior midgut showing disorganized protoplasm (PT) removed through the germination pore (P), forming a clear ring (EP). PW—pollen wall. Bar = 1 μm.



Figs. 3–5. Fig. 3, *Scaptotrigona postica*. Pollen grain inside of the posterior midgut showing disorganized protoplasm (PT) without extrusion. PW—pollen wall. Bar = 1 μm. Fig. 4, *Trigona spinipes*. pollen grain inside the posterior midgut showing disorganized protoplasm retracted in the center (arrow) leaving a clear peripheric space (CP). Bar = 1 μm. Fig. 5, *Trigona spinipes*. Material released by the pollen grains, showing lipid-like droplets (L) surrounded by electron dense material (ED). Bar = 1 μm.

origin. The pollenkitt is a protein- and lipid-rich layer that coats some species of pollen. Because the species of pollen grains present in the midgut of the stingless bees studied here could not be determined in order to know if they have or not pollenkitt, the origin of material outside of the pollen grains although undetermined, is though to be, at least partially resultant of pollen protoplasm extrusion.

Pollen grains with their content disorganized and retracted at the center have been also observed in adult bees by Cruz-Landim (1985), Klungness and Peng (1984b) and Cruz-Landim and Serrão (1994). As suggested by Klungness and Peng (1984b) pollen grains with these conditions have a wall composed primarily of cellulose and sporopollinin. Presumably, these species of pollen would be of less nutritive value to the bee. This is in agreement with Maurizio (1954) who observed that pollen of different species has different nutritional value to the worker of *Apis mellifera*.

Further, we believe that the larvae must profit from the ingested pollen, because of it lasting in the midgut during all larval life, given it needs a long time to be digested.

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